Remarks

Please note that the references to pages and line numbers herein refer to the amended specification that was submitted on October 15, 2001, rather than the specification that was originally filed.

The claimed invention

The claims as amended are drawn to mice and mouse cells whose genome is heterozygous or homozygous for a mutation engineered into the Erk5 gene. The Erk5 protein is a member of the mammalian MAP kinase family, an important group of molecules that play roles in a variety of processes ranging from the cell cycle to apoptosis. In addition, signal transduction via MAP kinase pathways has been implicated in a number of diseases and clinical conditions. In a homozygous state the mutation engineered into the genome of the mice and cells of the invention results in a functionally deficient Erk5 gene and embryonic death characterized by a lack of vasculature, revealing that Erk5 plays a role in angiogenesis.

Rejections under 35 U.S.C. § 112

Claims 3, 4, and 9 stand rejected under 35 U.S.C. § 112, first paragraph, on the ground that the specification, while being enabling for a homozygous transgenic mouse embryo, does not reasonably provide enablement for any non-human mammalian embryo whose genome comprises a mutation in the endogenous Erk5 gene. Claims 3 and 4 have been amended to recite a mouse embryo rather than a mammalian embryo. Claim 9 has been canceled. Withdrawal of the rejection is respectfully requested.

Claims 1, 2, 5-8, and 10-12 stand rejected under 35 U.S.C. § 112, first paragraph, on the ground that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. The Examiner asserts that "while the steps to produce knockout mouse have been well developed and used in mice, they have not been fully developed in other animals, particularly the art of gene targeting in ES cells and culture and selection of the ES cells that harbor the desired integration...". Claims 1, 2, 5-7, and 12 have been amended to recite "mouse" with respect to the embryo, cell,

or organism claimed. Claims 8, 10, and 11 have been canceled. Withdrawal of this rejection is respectfully requested.

The Examiner further asserts that claims 1, 2, 6-8, and 10 – 12 are not enabled with respect to a heterozygous mouse or to cells wherein one allele of the Erk5 gene is not mutated because the specification teaches that the "heterozygous mouse did not have any distinguishing phenotype and were normal (and)...an artisan would not know how to use these mice." and, similarly, "cells isolated from the heterozygous mice will not have any functional abnormality and therefore, an artisan will not know how to use these cells." As noted above, claims 1, 2, 6, and 7 have been amended and claims 8, 10, and 11 have been canceled, so these remarks will address the remaining claims as amended. Applicants respectfully disagree with the Examiner's assertion that the heterozygous mice and cells of the invention lack utility. It is evident that one can use the heterozygous mice to generate homozygous mice wherein both alleles of the Erk5 gene are mutated such that a phenotype results (i.e., a lack of vasculogenesis and angiogenesis), as described in the specification. For example, as stated at p. 7, lines 7 – 9, the heterozygous animals are interbred to obtain an embryo homozygous for a nonfunctional Erk5 gene. Thus one use of heterozygous mice wherein one allele of the Erk5 gene is not mutated is to generate homozygous mice wherein both alleles of Erk5 are mutated. In fact, given that the homozygous mice die as embryos, the heterozygous mice are particularly important as it will not be possible to produce homozygous mice directly as offspring from parental homozygotes. Furthermore, the heterozygous mice of the invention are useful for interbreeding with other mice, e.g., mice whose genome is heterozygous or homozygous for mutations in one of the other MAP kinase genes or other genes involved in a MAP kinase signaling pathway.

As described on p. 5, line 23 through p. 6, line 14, heterozygous mouse ES cells are useful to generate heterozygous mice, which are in turn useful for the generation of homozygous mice. In addition, as described on p. 7, line 29 through p. 8, line 9, cells that are heterozygous for a defective Erk5 gene can be used to generate cells which are homozygous for the Erk5 mutation, which can in turn be used to screen for compounds that are capable of rescuing or compensating for the defect in functional Erk5 expression, etc. (see p. 7, lines 19-23). Thus it is submitted that a utility for both heterozygous mice and heterozygous mouse cells wherein one

allele of the Erk5 gene is not mutated is to generate homozygous mice and cells wherein both alleles of Erk5 are mutated.

Furthermore, notwithstanding the fact that the heterozygous mice and heterozygous mouse cells appear phenotypically normal, they are useful to screen for compounds that increase Erk5 activity or compensate for a deficiency of Erk5. For example, as stated in the specification at p. 7, lines 3 - 6, cultures of heterozygous cells are "useful to assay for compounds that potentially rescue the Erk5 mutation". It will be appreciated that although the cells are phenotypically normal, since they have only one functional Erk5 allele the amount of Erk5 protein produced in the heterozygous cells will in general be approximately half the amount produced in wild type cells. As described on p. 2, lines 1-2, Erk5 is a kinase, i.e., a protein that transfers a phosphate group to other proteins. As such, the activity of Erk5 can be measured by performing a kinase assay, e.g., an *in vitro* kinase assay as mentioned on p. 11, lines 21-26 and described in Y. Kato, et al, EMBO J., 16, pp. 7054-66 (1997), which reference is incorporated into the present application by reference. As described in Kato at p. 7055, left column and in the figure legend to Figure 1, an *in vitro* kinase assay involves culturing cells that express a kinase of interest such as Erk5, isolating Erk5 protein from the cells, and measuring the ability of the isolated protein to phosphorylate a substrate. This assay can be performed in the presence of candidate compounds using cells that are heterozygous for an Erk5 mutation as a source of Erk5 protein. Erk5 activity in heterozygous cells in the presence of a candidate compound may be compared with Erk5 activity in wild type cells. Compounds whose effect is to increase the level of Erk5 activity in the heterozygous cells so that it more closely resembles the level of activity in wild type cells are selected as activators of Erk5 expression or activity. The specification describes a nonlimiting range of compound types that may increase Erk5 expression at p. 17, lines 20-30. As described on p. 17, lines 11-19, such compounds are useful for increasing angiogenesis in a patient in need thereof. As further described on p. 18, lines 5-14, increasing angiogenesis is useful in the treatment of a variety of conditions including diabetic neuropathic ulcers, wounds, other ulcers, ischemia, atherosclerosis, etc. It is noted that this example of the utility of cells heterozygous for a disruption of the Erk5 gene describes but one potential use of these cells.

One of ordinary skill in the art would recognize that the heterozygous mice are similarly useful. For example, candidate compounds can be administered to heterozygous mice, which are

then allowed to interbreed. If a candidate compound rescues the Erk5 mutation, then homozygous offspring of these mice will survive rather than dying prior to birth and/or will display a greater degree of vasculogenesis and angiogenesis than in the absence of the compound. As described on p. 6, line 28 through p. 7, line 6, heterozygous mice are also useful as sources of heterozygous cells, some of the uses of which are described above. In summary, applicants submit that based on the teachings of the specification and the knowledge of one or ordinary skill in the art, substantial and specific uses for both the heterozygous mice and heterozygous cells bearing a disruption in one allele of Erk5 are readily apparent.

Claims 1-5, 7-8, and 10-12 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner asserts that the specification has disclosed the phenotype of a homozygous mouse embryo but not the phenotypes of homozygous mammals of other species. As noted above, claims 1-5, 7, and 12 have been amended so that they are limited to mice, and claims 11 and 12 have been canceled. Withdrawal of this rejection is respectfully requested.

Claims 1, 2, and 6-12 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner states that claims 1 and 6 are vague and indefinite because the limitation "wherein a...embryo" does not limit the claims since the claims are drawn to a heterozygous mammal which cannot have this limitation. Accordingly, applicants have amended claim 1 to remove the phrase "in said embryo" that forms the basis for the rejection under 35 U.S.C. § 112, second paragraph. In amended claim 1 the phrase "wherein in a homozygous state said mutation results in a functionally deficient Erk5 gene and embryonic death characterized by a lack of vasculogenesis and angiogenesis" limits the mutation in the Erk5 gene, and the mutation in turn describes and limits the claimed mice. Similarly, applicants have amended claim 6 to remove the phrase "wherein a mammalian embryo whose genome is homozygous for said mutation is characterized by". In amended claim 6 the phrase "wherein in a homozygous state said mutation results in a functionally deficient Erk5 gene and a lack of vasculogenesis and angiogenesis and a failure to survive to birth limits the mutation in the Erk5

gene, and the mutation in turn describes and limits the claimed mice. Withdrawal of the rejection is respectfully requested.

The Examiner has stated that claims 2 and 7-12 are indefinite because they depend on claims 1 and 6. Applicants submit that the amendment of claims 1 and 6 renders claims 2, 7, and 12 definite. As noted previously, claims 8-11 have been canceled. Withdrawal of this rejection is respectfully requested.

In conclusion, in view of the amendments and remarks presented herein, the application and pending claims comply with the requirements of 35 U.S.C. §112. Applicants therefore respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful in resolving any remaining issues, the undersigned would greatly appreciate the opportunity to discuss such issues at the Examiner's convenience. The undersigned can be contacted at (617) 248-5000 or (617) 248-5071 (direct dial).

A check to cover the fee for a one month extension of time is enclosed. Please charge any additional fees associated with this filing, or apply any credits, to our Deposit Account No. 03-1721.

Respectfully submitted,

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